A Review of Analytical Methods in Investigations of Mutagens Associated with Air Particulate Material

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Many of the analytical methods used in environmental mutagenesis research are derived from techniques developed for the identification of specific, pre-determined, target analytes. However, the approach used in the determination of potential mutagens in complex environmental mixtures is fundamentally different. It is essential for effective strategies to combat the health effects of environmental mutagens to precisely characterize the nature of the compound, a mixture of compounds, or environmental factors that enhance or reduce mutagenicity. The combination of short-term bioassays with analytical chemical techniques has been successfully used in the identification of a variety of mutagenic compounds in complex mixtures. This marriage of chemical and biological tests is often referred to as "bioassay-directed chemical analysis" or "bioassay-directed fractionation"

Using bioassay-directed fractionation approaches, the crude extracts can be fractionated, and the resulting sub-fractions assayed to isolate mutagenic activity in mixtures of lesser complexity. Several iterative fractionation steps are usually required before mutagenic activity can be isolated in a manageable number of sub-fractions whose complexity is reduced to the point that structures for the candidate chemicals present can be postulated. Complete chemical confirmation of the proposed structures is obtained with authentic standards which may require custom synthesis. Verification of mutagenicity is necessary to confirm the biological activity of the compounds identified, their relative potencies, and their relative contributions to the overall potency of the matrix being examined. Subsequent to the confirmation of a causal mutagenic agent, more detailed studies can be conducted regarding such factors as persistence, exposure and metabolism.

A myriad of fractionation schemes have been developed for studies of mutagenicity of air particulate material. For the purposes of this review, many of these methods are discussed according to three general schemas for the processing of crude organic solvent extracts from air particulate samples: 1. acid/base/neutral partitioning followed by fractionation using open-column chromatography (silica/alumina) and/or HPLC; 2. fractionation based on NP-HPLC using a cyanopropyl or chemically-similar stationary phase, and; 3. fractionation by open-column chromatography followed by NP-HPLC using a cyanopropyl or similar stationary phase. In these cases, the HPLC methods may be preparative, semi-preparative, or analytical scale in nature, and can represent preparative and/or level 1 and higher fractionations. Variations based on acid/base/neutral partitioning followed by a chromatographic separation result in fractionation based both on acid/base/neutral and polarity characteristics, while the chromatographic methods result in separations based exclusively on polarity. Although these methodologies do not represent significant advances in technology over the past 30 years, their simplicity, low cost, effectiveness, and robustness combine to result in their continued application in modern analytical laboratories.